Characterization of *Lactobacillus brevis* with potential probiotic properties and biofilm inhibition against *Pseudomonas aeruginosa*

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Introduction

- Probiotics - live microbial supplement which improve hosts health by maintaining intestinal microbiota.
- Ideal probiotic - non-pathogenic, non-toxic, resistant to gastric acid, and produce antibacterial substances. (Gismondo et al.,1999)
- Lactic acid bacteria (LAB) strains used as Probiotics - *Lactobacillus acidophilus, L. lactis, L. casei S. thermophilus, L.bulgaricus.* (Ouwehand et al., 2002)
- Globally - Consumption of functional foods or nutraceuticals with potential probiotic microorganisms. (Quinto et al., 2014)
- *Lactobacilli* strains have been extensively studied in biofilm inhibition of pathogenic strains such as *Pseudomonas aeruginosa and Klebsiella pneumoniae.*

Methods

- Screening and Isolation: Isolation on Rogosa SL agar (Hartemink R, Domenech VR, 1997).
- Identification of the isolate: Based on gram staining, catalase test, biochemicals and 16S rRNA sequencing (Bergey's manual Volume 2).
- Determination of probiotic potentials of LAB: Tolerance to NaCl, bile, low pH and lysozyme, antibiotic susceptibility testing, cell surface hydrophobicity (Prabhurajeshwar, C. 2017).
- Antimicrobial activity of *L. brevis* against *S. aureus*, *E.coli*, *P. aeruginosa* , *K. pneumoniae* by scrape and streak method (Gillies, R. R., & Govan, J. R. 1966).
- Antibiofilm activity of *L. brevis* against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* determined by crystal violet assay (Mathkhury HJF.2011).
- Stability of *L. brevis* at 4°C and at 27°C.
- Stability in ice-cream by determining their viability onto MRS agar after 1 week incubation.

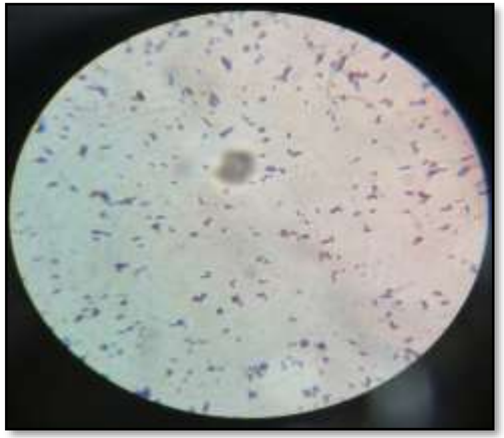
Results

Screening and Isolation of Lactic acid bacteria

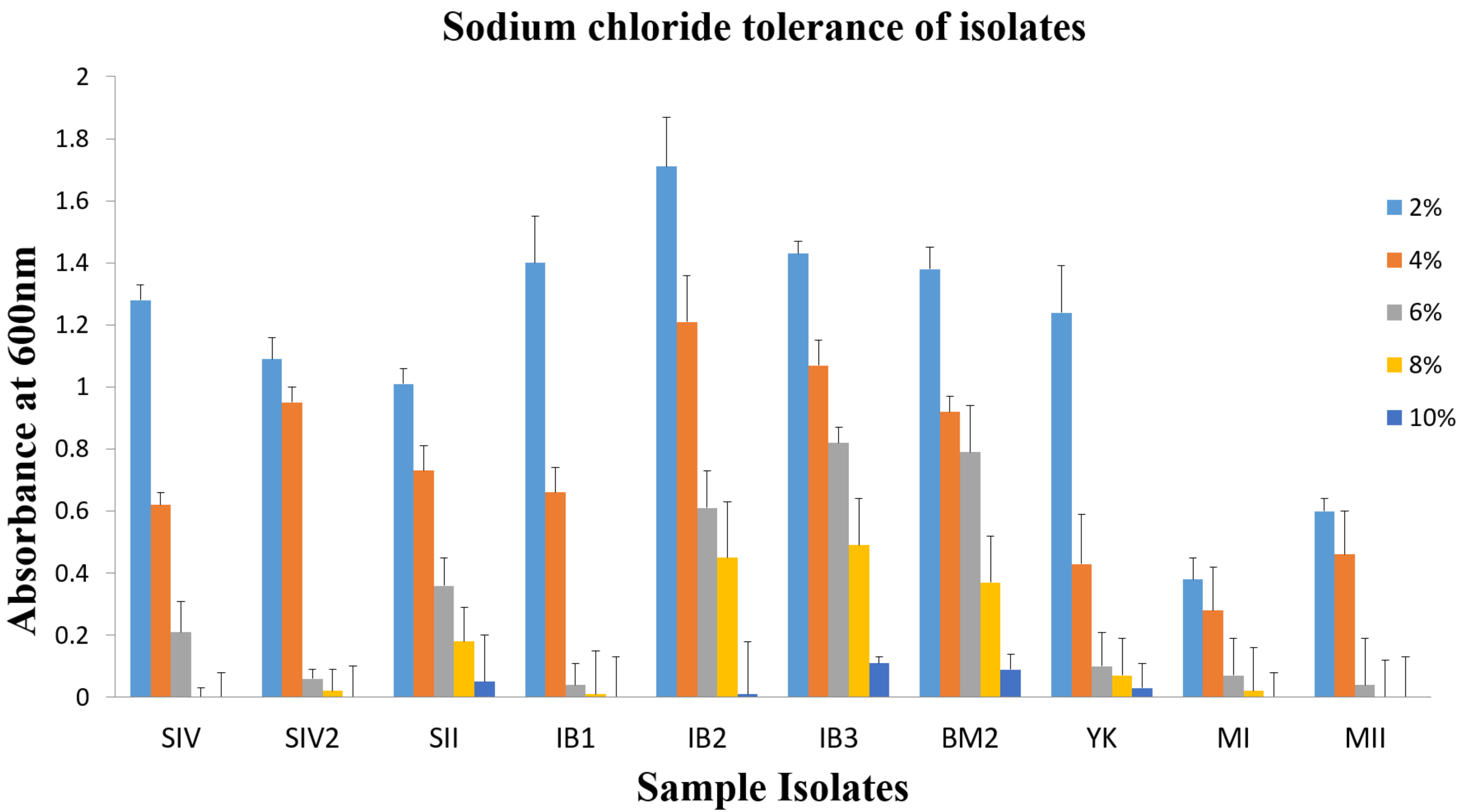
Ten isolates : MI, MII, BMI, SII, SIV, SIV2, IB1, IB2, IB3 and YK grew on Rogosa SL agar were selected for probiotic potential.

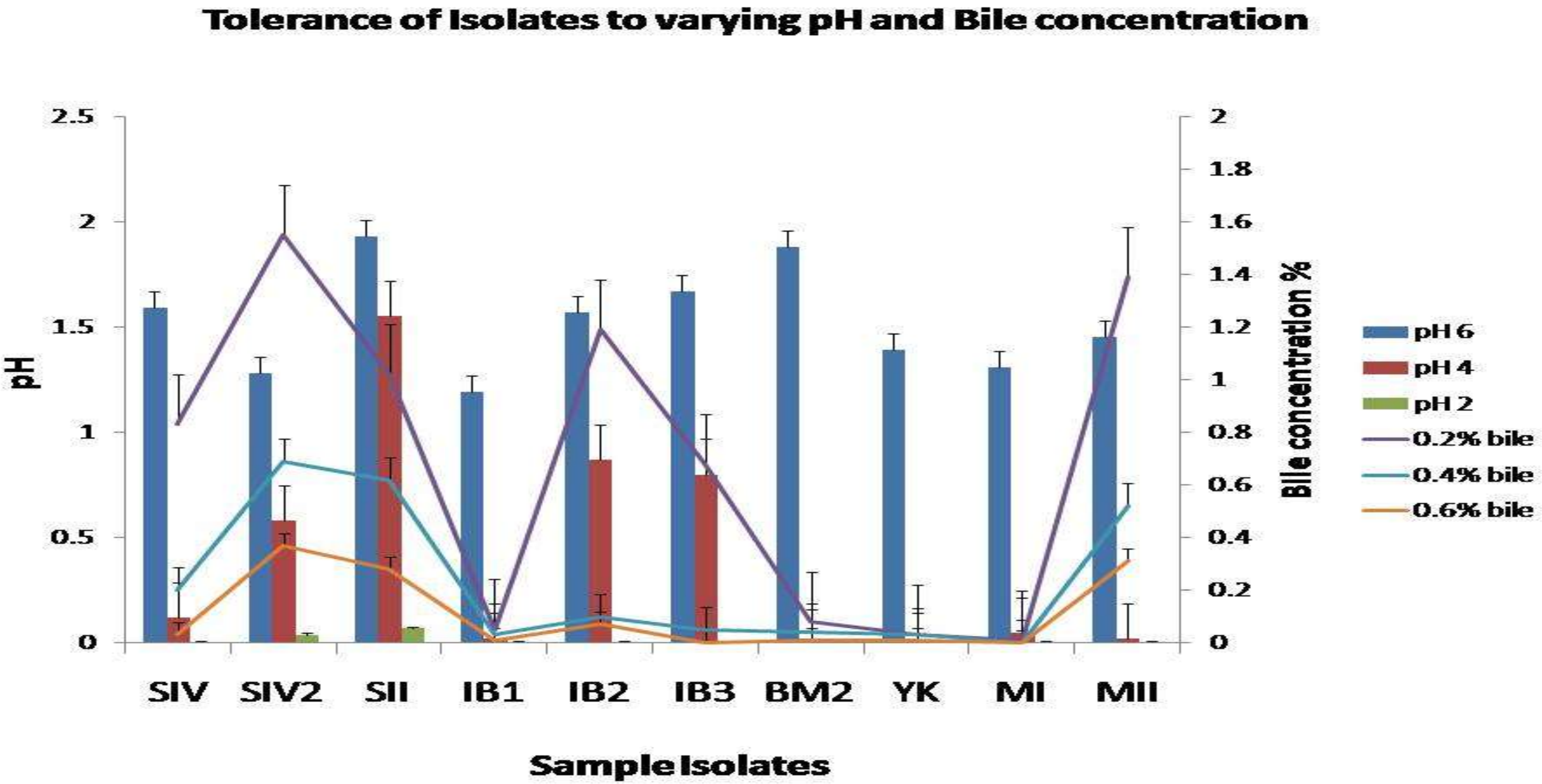
Biochemical identification: catalase, oxidase and in IMViC test negative.

White, circular, opaque colonies observed.



Gram positive short rods


Sodium chloride tolerance of isolates
Absorbance at 600nm
Sample Isolates
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
SIV
SIV2
SII
IB1
IB2
IB3
BM2
YK
MI
MII
2%
4%
6%
8%
10%



Maximum survival rate after exposure of 100ug/ml lysozyme – IB2(77.4%), SII (58.6%).

Isolate SII gave promising results as a candidate for "Probiotic" from the previous results.

Antibiotic susceptibility test of SII:

- Resistant towards amikacin, ciprofloxacin, gentamicin and vancomycin
- Very less sensitivity: ampicillin and tetracycline.

Cell surface hydrophobicity

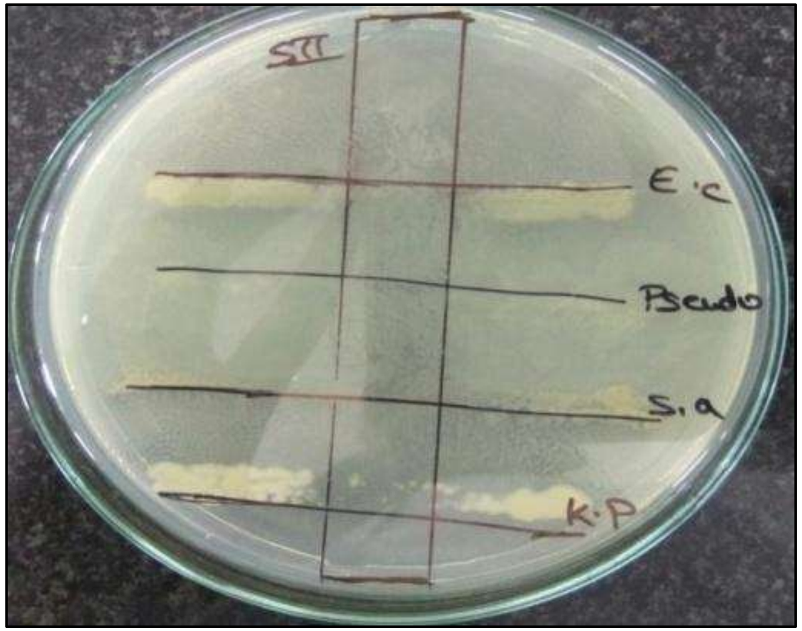
- Hydrophobicity % of SII to n-hexane was found to be **5.03%**

Antimicrobial activity

- Isolate SII showed inhibitory effect towards all indicator strains such as *E. coli*, *K. pneumoniae*, *S. aureus*, *P. aeruginosa*.

Stability

- SII was stable at lower temperatures(4 °C).



Antimicrobial activity of Isolate SII

Antibiofilm activity of SII

- Cell free extracts of SII inhibited biofilm formed by *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*

Indicator Strains	Absorbance at 620nm		
	Test (CSF)	Control	% Inhibition
Klebsiella pneumoniae	0.14	0.18	**22.2%**
Pseudomonas aeruginosa	0.09	0.19	**52.63%**

P. aeruginosa **biofilm inhibition by Isolate SII**

Molecular and genetic analysis of SII

- *Lactobacillus brevis ATCC 14869* – Accession no. NR_116238.

Discussion

- ***Lactobacillus brevis*** was isolated from a sauerkraut sample
- Tolerance to low pH, high NaCl concentration, growth in presence of bile – Survival in extreme conditions of intestinal tract (Graciela FVD, 2001)
- Lysozyme tolerance of *L.brevis* – Administered orally (Kimoto-Nira,Suzuki,2008).
- *L. brevis* resistance towards a broader range of antibiotics – Can be used in combination with antibiotics (Charteris et al., 1998).
- Inhibition mechanisms – bacteriocin, hydrogen peroxide production, lowering pH (Aween et al., 2012).
- Biofilm inhibition - Antimicrobial compounds in the cell free supernatant cause death of cells, rendering aggregation of cells (Ben S. et al 2013).

Discussion

- Cell surface hydrophobicity of *L. brevis* - Not a prerequisite for a strong adherence capacity (Vinderola et al., 2004).
- Stability of *L. brevis* - Refrigerating probiotic will maintain viability and shelf life. (Ananta et al., 2005).
- In other studies, it has been reported that *L. brevis* could be a promising isolate with respect to Probiotic, as well as biofilm inhibition towards other pathogens.
- *L. brevis* ATCC 8287 – promising candidate as a probiotic supplement in dairy product (Ronka E.2003).
- Bacteriocin produced by *L. brevis* DF01 – Inhibited biofilm formed by *E. coli* and *S. typhimurium* (Kim N, et.al 2018).
- Inhibitory effects of lactobacilli against *P. aeruginosa* and their biofilm formation were investigated (D Shorkri,et.al . 2017).
- *L. brevis* BBE-Y52 strain – potential to be used in oral care products (Fang F.2018).

Conclusion

- ***Lactobacillus brevis*** is a potential probiotic candidates which can be isolated from dairy food, fermented vegetables etc.

- Probiotics can be used to cure health issues as well as their products can be used in the pharmaceutical industry as well as in food industry.

Future Prospects

- Characterization of Antimicrobial substances produced by *L. brevis*.
- Biofilm inhibition activity towards oral pathogens and other strains.
- As a functional starter culture in dairy products like yoghurt and ice cream can be promising outcomes in the nearest future.

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THANK YOU

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